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THE EFFECT OF EXTERNAL ANIONS ON STEADY-STATE CHLORIDE EXCHANGE ACROSS ASCITES TUMOUR CELLS

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SUMMARY

1. The rate of cell chloride exchange, or efflux coefficient, was measured after equilibration in media of different anionic composition.

2. When sulphate substituted for chloride in the medium, the efflux coefficient was always higher than in control chloride solutions and varied inversely with external chloride concentration. In sulphate the chloride efflux coefficient varied from 19·6 to 100·7 hr^{-1}. The mean control efflux coefficient was $6{\cdot}60 \pm 0{\cdot}677$ (S.E. of mean).

3. In contrast, when external nitrate substituted for chloride, the efflux coefficient was independent of external chloride concentration and the same as in control chloride media. The mean value in nitrate was $6{\cdot}42 \pm 0{\cdot}603$ (S.E. of mean). The results confirm findings of Hempling & Kromphardt (1965).

4. Steady-state chloride *flux* varied in direct proportion to the external chloride concentration, which would be expected for passive chloride exchange. However, the slope of the line relating these variables was higher in sulphate than in nitrate and control media. Thus at any given external chloride concentration chloride flux was greater in sulphate than in nitrate and control solutions.

5. It is suggested that the effect of sulphate to increase cell chloride exchange may be related to its greater tendency to bind water, relative to chloride and nitrate.

INTRODUCTION

It has been postulated that chloride in the Ehrlich ascites tumour cell distributes passively and according to the electrical potential difference (PD) across the membrane (Grobecker, Kromphardt, Mariani & Heinz, 1963). Measurements of the PD with micro-electrodes were consistent with this view although it was suggested that sodium as well as chloride con-

tributed to the potential (Aull, 1967). In the micro-electrode study the PD was measured soon after mixing cells with sulphate or nitrate solutions as well as in the control chloride solutions. It was found that in sulphate the cells depolarized or even reversed polarity but in nitrate they showed only slight depolarization. Therefore, it was important to learn more about the membrane permeability to chloride in these different anionic environments.

A report of chloride exchange in nitrate solutions by Hempling & Kromphardt (1965) indicated that the chloride efflux coefficient remained quite constant at different external chloride concentrations. In the present study steady-state chloride exchange at 22–25° C was measured as a function of external chloride concentration, and a comparison was made between the use of sulphate and nitrate as the replacing anions for chloride. It was found that the rate of cell chloride exchange (efflux coefficient) increased markedly when chloride was replaced with sulphate and in inverse proportion to the external chloride concentration. In contrast, the chloride efflux coefficient in nitrate media was the same as in control chloride and was independent of the external chloride concentration, confirming the results of Hempling & Kromphardt. The effects of the external anions might be related to their relative hydration. This in turn may influence constituents of the cell membrane which are important for chloride transfer.

METHODS

Experimental design

1. *Chloride exchange from tracer uptake studies.* Suspensions of Ehrlich ascites tumour cells were obtained from male Swiss white mice, strain HA/ICR, bearing growths 6–13 days old. The cell suspensions were aspirated from the peritoneal cavity and were usually bloody. They were cleaned by gentle centrifugation and resuspension in stock chloride Ringer (see Materials, 'experimental media'). After one or two washes with stock chloride Ringer, the cells were washed two or three times with the specific experimental solution to be used. All studies were carried out at room temperature (22–25° C) and in air.

Cells were equilibrated with their environments at a concentration of $0{\cdot}5–1{\cdot}0 \times 10^7$ cells/ml. for 45–60 min. At the end of this period the cell chloride content was at steady state and ^{36}Cl was added to measure chloride flux. ^{36}Cl was obtained from New England Nuclear, Boston, Mass. as 2·8 N-HCl and was neutralized with NaOH. The final ^{36}Cl concentration in the suspension was 0·25–0·5 μc/ml. Samples of 10 ml. were removed as soon as possible after mixing with ^{36}Cl and at intervals thereafter for 1 hr. The samples were centrifuged at maximal speed on the International centrifuge (2100 *g*) for 30 sec. Supernatant was discarded and the remaining packed cell pellet was washed and resuspended two times in 20 volumes of ice-cold, isosmotic dextrose (57 g/l., buffered to pH 7·4 with 0·167 M tri (hydroxymethyl) amino methane (Tris)-acetate). At the end of the sampling period, an aliquot of supernatant environment was saved. All samples were analysed for Cl, Na, K, and ^{36}Cl.

2. *Rapid sampling of sulphate suspensions.* Flux determinations in sulphate media showed that unidirectional chloride movements were too rapid to measure accurately

by the above method. Therefore, a technique was used in which samples were obtained at short intervals after mixing cells with ^{36}Cl. The technique was similar to one used by Aull & Hempling (1964) for sodium fluxes and by Levinson & Blumenson (1970) for calcium fluxes. A summary is given in the flow diagram (Fig. 1). A concentrated cell suspension ($1-3 \times 10^7$ cells/ml.) was maintained under 100 % O_2 in a 500 ml. Erlenmeyer flask. An aliquot of 2 ml. was removed to a centrifuge tube, and mixed well with 100 μl. ^{36}Cl (50 μc/ml.). After 25–30 sec, ice-cold dextrose was added and the cell sample was centrifuged and washed in the usual way. Subsequent aliquots

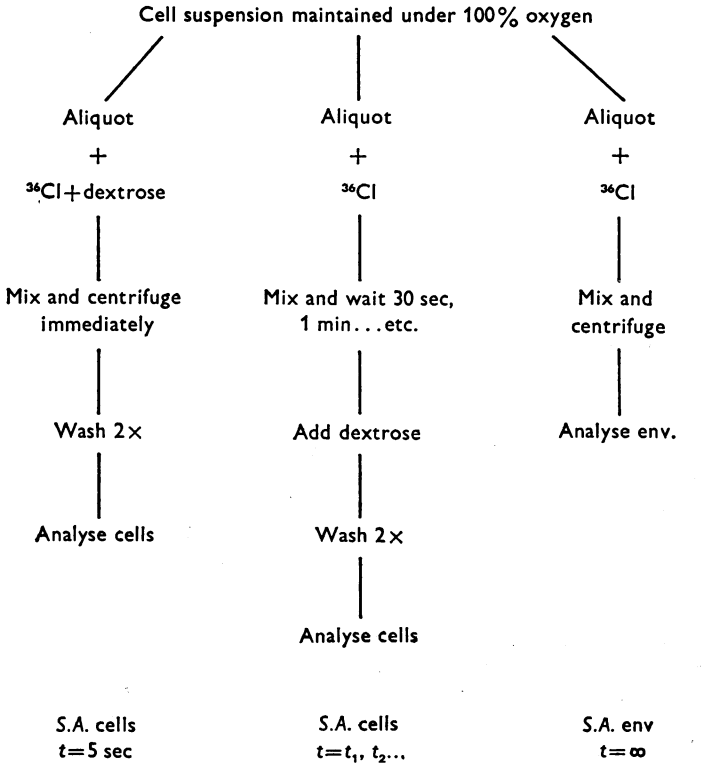

Fig. 1. Flow diagram of procedure to study steady-state chloride exchange in chloride-sulphate media. See Methods section on rapid sampling of sulphate suspensions.

were handled in the same way but the interval between adding ^{36}Cl and centrifuging with dextrose was varied. In this manner samples could be obtained, at, for example, 30 sec, 1 min, 1½ min, etc. (Fig. 1, middle column). To obtain an environment sample, the supernatant from a mixed aliquot was saved (Fig. 1, last column). In addition, as indicated in the first column of Fig. 1, a cell sample was mixed with ice-cold dextrose containing ^{36}Cl and then washed in the usual way (t = 5 sec). The amount of ^{36}Cl contained in the washed cell pellet represented contaminating ^{36}Cl from the environment plus any ^{36}Cl that entered the cells during the few seconds between adding ice cold dextrose and centrifuging. This radioactivity presumably was present in all other cell pellets of the same experiment. The entire sampling period actually

lasted about 50 min. Analysis for cell chloride content at the beginning and end of the 50 min period showed that the cells essentially were in a steady state over this interval. Sometimes there were small net movements of chloride but they never exceeded 1 % of the unidirectional chloride flux and usually were much lower.

Materials

1. *Experimental media.* The chloride concentration of the experimental media was varied by mixing a stock chloride Ringer, the control solution, with varying proportions of either a stock sulphate Ringer or a stock nitrate Ringer. The stock solutions were made as follows: *stock chloride (control) Ringer* (Hempling, 1958) = 9 g NaCl, 40 ml. 0·154 M-KCl, 15 ml. 0·11 M-NaH_2PO_4, 85 ml. 0·11 M-Na_2HPO_4, to 1 l. with distilled H_2O; *stock nitrate Ringer* = 13 g $NaNO_3$, 40 ml. 0·154 M-KNO_3, 15 ml. 0·11 M-NaH_2PO_4, 85 ml. 0·11 M-Na_2HPO_4, to 1 l. with distilled H_2O; *stock sulphate Ringer* = 300 ml. stock sucrose solution (41·56 g sucrose, 4 ml. 0·154 M-K_2SO_4, 6 ml. 0·167 M-Tris HCl at pH 7·63, to 400 ml. with distilled H_2O) plus 700 ml. stock sulphate solution (860 ml. 0·13 M-Na_2SO_4, 20 ml. 0·154 M-K_2SO_4, 15 ml. 0·11 M-NaH_2PO_4, 85 ml. 0·11 M-Na_2HPO_4, 20 ml. stock sucrose solution). These solutions had pHs of 7·36–7·47, osmolarities of 305–325 m-osmole/l. and contained 172 mM-Na and 6 mM-K. Generally, the medium chloride concentration was varied by mixing 1 part chloride stock (160 mM-Cl) with 1, 2, 3, 4, or 7 parts of either nitrate or sulphate stock (0 mM-Cl). The final chloride concentration of the medium was determined by chemical analysis of the supernatant fluid from an experimental cell suspension.

2. *Analytical methods.* The packed, washed cell pellets were extracted with 4·5 ml. cold distilled water and 0·5 ml. 35 % (v/v) cold perchloric acid. The extract was analysed for sodium and potassium with a Baird flame photometer, and for chemical chloride with a Buchler–Cotlove electrometric titrator at low rate. (The perchloric acid extraction procedure gives the same results for cell sodium and potassium content as the distilled water extraction used by Hempling (1962), Kromphardt (1968) and others. However, the chloride content is some 20 % less with perchloric acid extraction and, with this technique, cell chloride is found to exchange completely with radioactive chloride in the environment (Simonsen & Nielsen, 1971). I am grateful to Harold G. Hempling for bringing this to my attention.) Supernatant environments were similarly analysed.

Cell water was measured by determining wet and dry weight of cells packed for 20 min by centrifugation at 2100 g (Aull, 1967). Correction was made for a trapped water volume of 0·20 ml./g wet cell weight, according to Levinson (1970).

^{36}Cl was determined by channels ratio counting on a Nuclear–Chicago liquid-scintillation counter. The scintillation mixture was 1 l. dioxane, 100 g naphthalene, 7 g 2,5-diphenyloxazole (PPO), plus 30 mg *p*-bis [2-(5-phenyloxazole)] 1-benzene (POPOP). To 10 ml. of this fluid, 0·5 ml. perchloric acid extract were added for determination of cell radioactivity. For environmental radioactivity, cell sample supernatant was diluted with water and perchloric acid to give the same counting rate and perchloric concentration as in the cell samples. Each sample was counted for a total of at least 1000 counts and the sample to background ratio was 100–300.

Cell chloride, sodium, potassium and water are expressed per 10^7 cells. Cell counts were done on a Neubauer–Levy haemocytometer and 1000 cells were counted. Osmolarity was measured on an Osmette osmometer, pH on a Fisher Accumet pH meter.

Calculations

The rate of chloride exchange was measured by isotopic uptake in the steady-state and the kinetics of a two-compartment closed system were applicable. The environment is a large compartment with constant specific activity. The equation describing the relation between the rate coefficient for cell chloride exchange, or efflux coefficient and the rate of increase of cell specific activity has been given by Solomon (1949), Sheppard & Martin (1950) and Wieth (1970).

Thus

$$\ln 1 - \frac{S.A._t}{S.A._\infty} = -bt + y,$$

$S.A._t$ = specific activity of cells at time, t.
$S.A._\infty$ = specific activity of environment or cells at isotopic equilibrium.
y = y intercept. At $t = 0$, y should be zero. In the experiments reported, the intercept at $t = 0$ did not differ significantly from zero, when analysed statistically (Tables 1 and 3).

$$b = K_e \frac{S_e + S_c}{S_e}$$

and

$$K_e = b \frac{S_e}{S_e + S_c},$$

where K_e is the efflux rate coefficient, S_e is the compartment size of environment chloride and S_c is the compartment size of cell chloride.

In the experiments reported here, $S_e/S_e + S_c$ was 0·98–1·00, so that K_e was taken as the regression coefficient (b) of the least squares regression equation for each experiment. The correlation coefficients for all these regressions were significant (25 out of 31 significant at 1 % level and 6 at 5 % level).

RESULTS

Cell chloride turnover in sulphate media

Initial studies of steady-state chloride exchange showed that in a sulphate-chloride environment the cells took up ^{36}Cl very rapidly and at a higher rate than in a nitrate–chloride environment. A comparison of the rate of uptake in the two media is shown in Fig. 2. The increase of cell chloride specific activity with time is given for cells suspended in sulphate and nitrate solutions having the same chloride concentration of 88 m-equiv/l. The half-times estimated from Fig. 2 are 1 min in sulphate and 14 min in nitrate. It is evident that cells reach an isotopic steady-state much more rapidly in sulphate than in nitrate.

The rate of uptake in sulphate was so rapid that it was necessary to apply a special technique to measure the chloride efflux coefficient. With this technique, samples could be taken at short intervals after mixing cells with ^{36}Cl. The procedure is outlined in Fig. 1, and described in Methods ('rapid sampling of sulphate suspensions'). The efflux coefficient was determined for cells equilibrated in sulphate media over a range of chloride

concentrations, from 38 to 134 m-equiv/l. In one instance, the chloride exchange of cells equilibrated with *control* chloride Ringer was determined by the rapid sampling method (Table 2, Expt. 5). The resulting value of the efflux coefficient was comparable to other controls determined by the usual slow sampling procedure [7·43 hr^{-1} compared to a mean of 6·60 ± 0·677 (S.E. of mean)]. Therefore the rapid sampling method in itself did not alter the chloride exchange characteristics of the cells.

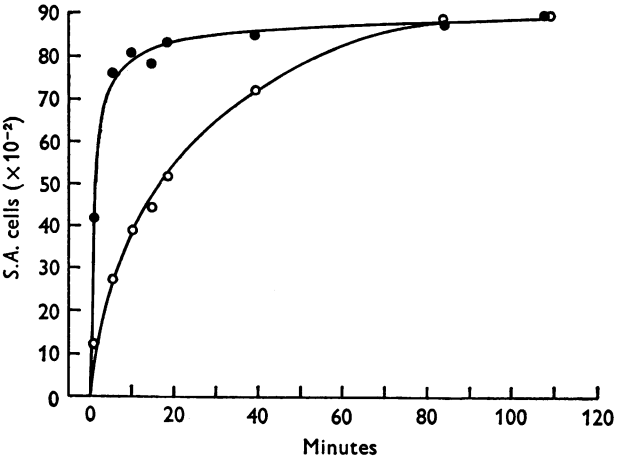

Fig. 2. Uptake of ^{36}Cl in the steady-state after equilibration in sulphate-chloride (●) or nitrate-chloride (○) media. Ordinate: specific activity of cells in cpm/μ mole. Environment chloride concentration was 88 m-equiv/l.

The semilog graphs for efflux coefficients of the same cell population at two different chloride concentrations are shown in Fig. 3. In each case the points fall neatly on a single line, indicating that the technique provides a simple means of obtaining efflux coefficients at high chloride turnover. The regression lines do not go exactly through zero $[1-(S.A._{t=0}/S.A._{\infty})\neq 1]$, but the difference from zero is not statistically significant, as shown in Table 1, 3*a* and 3*b*. Table 1 also shows that this was true for all of the sulphate experiments.

Fig. 3 demonstrates that in sulphate at 84 m-equiv/l. outside chloride, the efflux coefficient was 63·6 hr^{-1} and at 58 m-equiv/l. the rate coefficient was 100·7 hr^{-1}. In contrast, the mean efflux coefficient in control solutions at 157 m-equiv/l. was only 6·60 ± 0·677 (S.E. of mean) hr^{-1} (see below). Thus, cell chloride turnover in sulphate-chloride experiments was an order of magnitude higher than that of cells in control chloride media.

Cell chloride turnover in sulphate was higher at the lower external chloride concentrations (higher external sulphate concentrations). This

TABLE 1. Statistics of intercept with ordinate in sulphate experiments. The intercepts were calculated from the least squares regression lines of $\ln [1-(SA_t/SA_\infty)] = -bt+y$. P is probability that the calculated value of the intercept at $t = 0$ differs from zero $[1-(S.A._{t=0}/S.A._\infty) \neq 1]$ due to random deviation

Expt. no.	External chloride (m-equiv/l.)	$1-\frac{S.A._{t=0}}{S.A._\infty}$	P
1*a*	134	0·970	$0{\cdot}4 < P < 0{\cdot}5$
b	121	1·090	$0{\cdot}4 < P < 0{\cdot}5$
2*a*	103	1·090	$0{\cdot}05 < P < 0{\cdot}1$
b	53	1·099	$0{\cdot}7 < P < 0{\cdot}8$
c	38	0·810	$0{\cdot}4 < P < 0{\cdot}5$
3*a*	84	1·072	$0{\cdot}05 < P < 0{\cdot}1$
b	58	1·130	$0{\cdot}2 < P < 0{\cdot}3$
c	41	0·690	$0{\cdot}5 < P < 0{\cdot}6$
4*a*	100	0·997	$P > 0{\cdot}9$
b	95	1·160	$0{\cdot}5 < P < 0{\cdot}6$
c	87	1·062	$0{\cdot}4 < P < 0{\cdot}5$
d	61	0·880	$0{\cdot}3 < P < 0{\cdot}4$
5*b*	105	0·890	$0{\cdot}8 < P < 0{\cdot}9$

TABLE 2. Chloride efflux coefficient as a function of external chloride concentration, using sulphate to replace chloride in the medium. Each numeral refers to a different cell population. The letters refer to an experimental variable, external chloride concentration

Expt. no.	External* chloride (m-equiv/l.)	External† sulphate (m-equiv/l.)	Efflux coefficient (hr^-)
1*a*	134	20	28·8
b	121	40	61·5
2*a*	103	40	38·4
b	53	61	86·9
c	38	70	46·1
3*a*	84	40	63·6
b	58	61	100·7
c	41	70	83·1
4*a*	100	20	27·7
b	95	70	73·0
c	87	40	75·6
d	61	61	61·4
5*a*	181	0	7·4
b	105	40	19·6

* Analytically determined on cell suspension supernatant fluid.
† Calculated concentration in suspending medium.

relationship was approximately true for any given cell population, as shown in Table 2. Each experiment number in Table 2 corresponds to a single cell population studied at several different external chloride con-

centrations. When the data of Table 2 are graphed together in the mass plot of Fig. 4, the increase in cell chloride exchange with decreasing outside chloride, or with increasing outside sulphate, is seen clearly. The

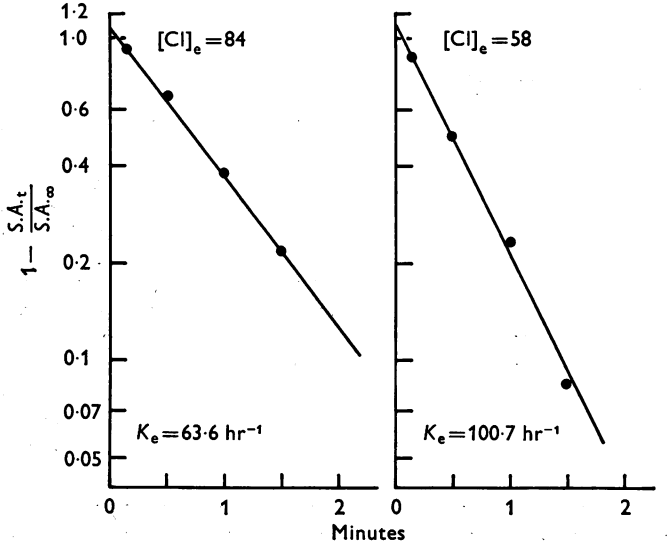

Fig. 3. Chloride efflux coefficient (K_e) of cell equilibrated in chloride-sulphate media at two different external chloride concentrations. Left graph: external chloride 84 m-equiv/l.; right graph: external chloride 58 m-equiv/l. Details of how K_e is determined are given in Methods (calculations).

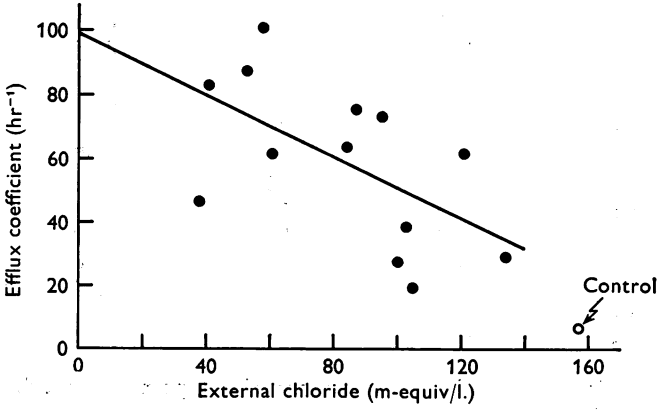

Fig. 4. Chloride efflux coefficient as a function of external chloride concentration when sulphate replaced external chloride. Experimental points (●) are those listed in Table 2 (excluding 5*a*). The equation of the mean squares regression line is: $y = -0·481X + 98·9$. Correlation coefficient is $-0·582$. The arrow points to the mean control efflux coefficient (○).

points can be fitted by a straight line having a correlation coefficient which is significant at the 5% level. In order to emphasize that the cell chloride turnover in sulphate was always higher than in control chloride media, the mean control efflux coefficient is included for reference.

Cell chloride turnover in nitrate and control media

Studies were done to compare cell chloride exchange in sulphate-chloride media with the exchange in nitrate-chloride solutions. The striking rapidity of cell chloride turnover in sulphate is emphasized further by the results of the nitrate experiments. Fig. 5 shows semilog plots for efflux coefficients of nitrate equilibrated cells. Whereas the external chloride *concentrations* were essentially the same as in the sulphate experiment of Fig. 3, the *rate coefficient* in nitrate was only 1/10 that in sulphate at the same external chloride concentration.

TABLE 3. Statistics of intercept with ordinate in nitrate experiments

Expt. no.	External chloride (m-equiv/l.)	$1-\frac{S.A_{t=0}}{S.A._{\infty}}$	P
1*e*	157*	0·782	$0{\cdot}05 < P < 0{\cdot}1$
f	37	0·870	$0{\cdot}1 < P < 0{\cdot}2$
g	25	0·920	$0{\cdot}2 < P < 0{\cdot}3$
2*e*	134*	1·021	$P > 0{\cdot}9$
f	74	0·950	$0{\cdot}4 < P < 0{\cdot}5$
3*e*	78	0·930	$0{\cdot}5 < P < 0{\cdot}6$
f	16	1·011	$0{\cdot}5 < P < 0{\cdot}6$
4*e*	82	0·940	$0{\cdot}7 < P < 0{\cdot}8$
f	58	0·920	$0{\cdot}6 < P < 0{\cdot}7$
g	23	0·880	$0{\cdot}05 < P < 0{\cdot}1$
5*a*	181*	0·956	$0{\cdot}3 < P < 0{\cdot}4$
6*e*	155*	0·760	$0{\cdot}05 < P < 0{\cdot}1$
f	83	0·990	$P > 0{\cdot}9$
g	29	0·880	$0{\cdot}1 < P < 0{\cdot}2$
7*e*	102	0·930	$0{\cdot}05 < P < 0{\cdot}1$
f	67	1·120	$0{\cdot}7 < P < 0{\cdot}8$
8*e*	152*	0·980	$0{\cdot}8 < P < 0{\cdot}9$
9*e*	164*	0·860	$0{\cdot}05 < P < 0{\cdot}1$

* Control chloride Ringer.

The rate of cell chloride turnover was measured in control solutions containing chloride at about 160 m-equiv/l., as well as in lower outside chloride concentrations in which chloride had been replaced with nitrate. The results showed that the rate coefficient of cell chloride exchange was the same whether chloride alone or different mixtures of nitrate with chloride were present in the external medium. Table 3 demonstrates that the y

intercept of the log regression lines was not significantly different from zero in any of the chloride or nitrate experiments (Fig. 5 shows experiments 4*e* and 4*f*).

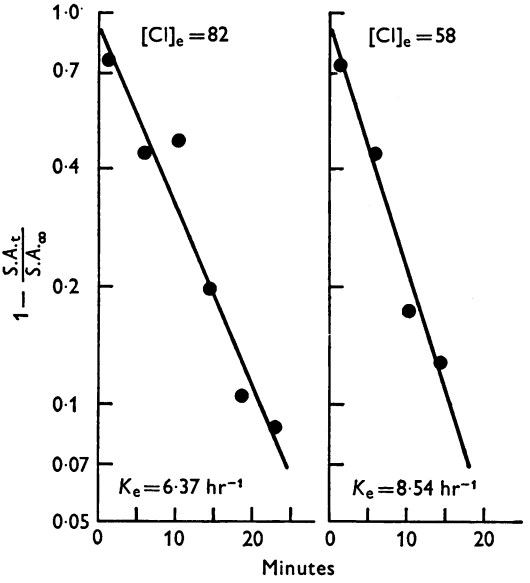

Fig. 5. Chloride efflux coefficient of cells equilibrated in chloride-nitrate media at two different external chloride concentrations. Left graph: external chloride 82 m-equiv/l.; right graph: external chloride 58 m-equiv/l.

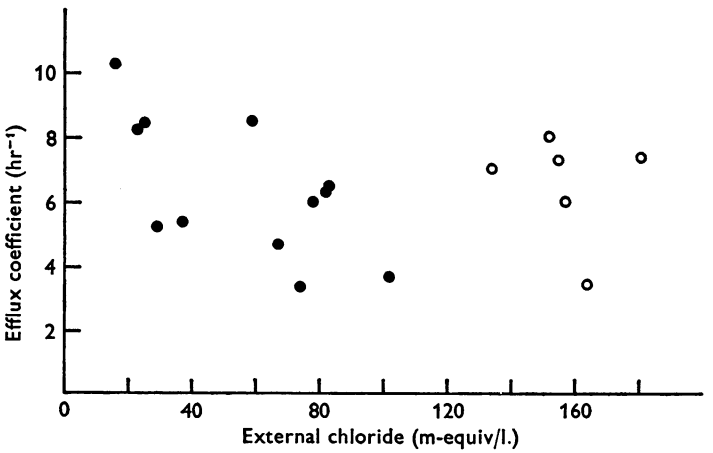

Fig. 6. Chloride efflux coefficient as a function of external chloride concentration when nitrate replaced external chloride. Control chloride without nitrate (○) and chloride-nitrate (●). There is no significant difference between the mean efflux coefficients of the two groups.

Fig. 6 is a mass plot of efflux coefficient as a function of outside chloride concentration. Control experiments are shown distinct from nitrate experiments but there is no significant difference between the two groups ($0{\cdot}8 < P < 0{\cdot}9$). The mean control efflux coefficient was $6{\cdot}60 \pm 0{\cdot}677$ (S.E. of mean) hr^{-1} and the mean efflux coefficient in nitrate, over the range 16–102 m-equiv/l. outside chloride, was $6{\cdot}42 \pm 0{\cdot}603$ (S.E. of mean) hr^{-1}.

The conclusions are that cell chloride exchanges at the same rate in chloride or nitrate environments and that cell chloride exchange is independent of external chloride concentration when chloride is replaced with nitrate. These conclusions contrast sharply with those drawn from sulphate experiments. Sulphate increased the turnover of cell chloride markedly, and in inverse relation to the medium chloride concentration (Fig. 4).

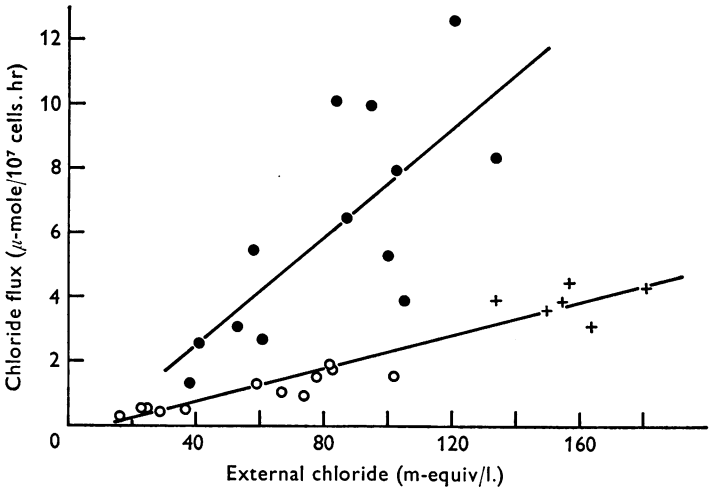

Fig. 7. Steady-state chloride flux as a function of external chloride concentration. Sulphate containing media (●), nitrate containing media (○), control media (×). Sulphate points can be fitted by the regression line: $y = 0{\cdot}0831x - 0{\cdot}808$. Correlation coefficient is 0·7339. Nitrate and control points can be fit by the regression line: $y = 0{\cdot}026x - 0{\cdot}33$. Correlation coefficient is 0·9572.

Chloride flux as a function of outside chloride

It has been concluded from a number of studies that chloride transfer in the ascites cell is passive in nature. One line of evidence has been the demonstration that the initial influx of chloride was directly proportional to external chloride concentration (Grobecker *et al.* 1963). In the present study, done in the steady-state, influx = efflux = efflux coefficient (hr^{-1}) × internal chloride content (μ-mole/10^7 cells). If the efflux coefficient were constant and the internal chloride content varied directly with

extracellular chloride concentration, a direct proportion between external chloride concentration and flux would be found. However, the present work showed that in sulphate environments, the efflux coefficient was *not* constant at different external chloride concentrations. Therefore it was important to determine the relationship between chloride *flux* and external chloride in sulphate experiments, and to compare the results with data from nitrate experiments. The results of such a study are given in Fig. 7 in which data are presented from all of the experiments reported in this paper. The data can be fitted by two lines having different slopes (correlation coefficients significant at 1 % level). One line with a larger slope describes the relation between flux and outside chloride in sulphate equilibrated cells. A different line with smaller slope describes the relation for nitrate and chloride (control) equilibrated cells. The lines as drawn do not go through zero but the intercepts do not differ significantly from zero when a statistical analysis is done (for sulphate, $P = 0{\cdot}7$; for nitrate $0{\cdot}1 < P < 0{\cdot}2$). Thus there is a direct proportion between steady-state flux and outside chloride concentration, reconfirming the concept of passive chloride transfer. However, at any given external chloride concentration, the flux is higher in sulphate than in nitrate, again demonstrating the effect of sulphate to accelerate chloride transfer.

DISCUSSION

Effect of external sulphate

The striking finding of this study of the ascites tumour cell was the effect of external sulphate to increase the rate coefficient of cell chloride exchange (efflux coefficient). This contrasted with the absence of an effect of external nitrate on cell chloride turnover. Hempling & Kromphardt (1965) also observed that tumour cell chloride turnover was independent of external chloride in nitrate media. They concluded from this that chloride exchange diffusion was absent in these cells.

The present study supports conclusions of other investigators (Grobecker *et al.* 1963; Kromphardt, 1968) that chloride transfer across ascites cells is passive. As shown in Fig. 7, steady-state chloride *flux* varied in direct proportion to the outside chloride concentration over a wide range. This relationship existed in sulphate as well as in nitrate or chloride environments, but the fluxes were always greatest in sulphate at any value of outside chloride.

The primary question arising from the work reported here concerns the mechanism by which sulphate acts to increase chloride exchange. One possible explanation would be that sulphate increases the permeability of the ascites cell membrane to all ions non-specifically. Experiments

bearing directly on this point are lacking but there is indirect evidence which shows that the membrane does not become abnormally permeable to sodium and potassium in the presence of sulphate. Table 4 compares the sodium and potassium concentration ratios established by the cells after equilibration with sulphate-chloride media with the corresponding concentration ratios in control media. The mean values of these cation gradients in sulphate media are quite comparable to the values seen in the one control experiment for which data are available from this study, as well as to the control values given by Hempling (1958) in a different study.

It is reasonable to conclude from these figures that the normal cation concentration gradients are maintained in spite of a five-to sixfold increase of cell chloride turnover. Therefore, non-specific membrane leakiness to all ions in the presence of sulphate seems unlikely. On the other hand, the exchange of anions other than just chloride may be increased in sulphate environments. Thus Salvatore, Salvatore & Wolff (1966) studied iodide transfer across the ascites cell and found that when sulphate replaced chloride in the environment, the cell/medium ^{125}I distribution increased rapidly to twice the control values (temp. 38° C). However, the ^{22}Na distribution was unchanged in the presence of sulphate, consistent with present findings.

Effect of PD

A study of the ascites cell PD with micro-electrodes demonstrated that when external chloride was replaced by sulphate solutions and the PD was measured within 10 min, the cells depolarized toward zero or sometimes showed small positive PDs (Aull, 1967). This was consistent with reversal of the chloride distribution ratio in the presence of a poorly penetrating anion. Such results might account for the rapid chloride efflux observed in this study if they were seen also in cells equilibrated for 1 hr or more in sulphate.

Systematic micro-electrode measurements have not been done in sulphate equilibrated cells but one can speculate that the value of the PD will depend at least partially on the sulphate permeability of the cells (Cotterrell & Whittam, 1971). This is known to be low relative to chloride but not negligible (Heinz & Mariani, 1957; Aull, 1967; Levinson, 1970). Further, the chloride concentration ratios determined in sulphate equilibrated cells and listed in Table 4 would give a negative cell PD relative to the environment. It is quite possible therefore, that the cells repolarize during equilibration as they have been observed to do in nitrate media (Aull, 1967) and as L cells have been seen to do in sulphate (Lamb & MacKinnon, 1971). If that is the case the increased chloride flux in sulphate would relate to factors other than the PD.

TABLE 4. Concentration ratios of sodium, potassium, and chloride across sulphate equilibrated cells and controls

$[\ ]_c$ = μ-mole/kg cell H_2O
$[\ ]_e$ = μ-mole/l. env

Expt. no.	Environment	External chloride (m-equiv/l.)	Chloride efflux coefficient (hr^{-1})	$\frac{[Na]_c}{[Na]_e}$	$\frac{[K]_c}{[K]_e}$	$\frac{[Cl]_c}{[Cl]_e}$
1*a*	Sulphate-chloride	134	28·8	0·137	28·1	0·124
2*a*	Sulphate-chloride	103	38·4	0·103	17·3	0·159
4*a*	Sulphate-chloride	100	27·7	0·186	22·3	0·111
Mean ± S.E.				0·142 ± 0·024	22·6 ± 3·1	
1*e*	Control-chloride	157	6·1	0·113	20·2	0·326
Hempling (1958)	Control-chloride	—	—	0·162	20·3	—
Levinson (1970)	Control, phosphate	—	—	—	—	0·365

Sulphate, chloride and nitrate as part of a lyotropic series

It has been noted that sulphate markedly increased parameters of chloride exchange when compared to nitrate and chloride. It is tempting to speculate that these three ions form part of a lyotropic series based on the relative tendency of the anions to bind water, and that their effects on chloride transfer in the ascites cell are somehow the result of this tendency. Thus a lyotropic anion series is, in order of increasing hydration (Giese, 1966):

SCN I NO_3 Br Cl acetate SO_4 tartrate citrate.

From the present study one might conclude that more hydrating anions like sulphate make the ascites cell membrane more permeable to chloride. This hypothesis must be subjected to further experimental verification, but preliminary work lends support to this view. It was found that another hydrating anion, divalent phosphate (Dowben, 1969), markedly increased ^{36}Cl uptake by ascites cells when present in the environment at concentrations higher than in control media (F. Aull, unpublished).

Salvatore and co-workers (1966) showed that the transfer of another anion, iodide, is similarly increased in sulphate. The effects of anion hydration may therefore apply more generally to anion exchange in the ascites cell.

Replacement of external chloride by other anions has been done in a number of systems, particularly in skeletal muscle and red blood cells. The work of Kenny, Spurway & Stenhouse (1970) is particularly relevant to the present study. They found increased efflux of ^{36}Cl from frog skeletal muscle in *fluoride* media and postulated that the effect was based on the high hydration energy of fluoride. They interpreted the results in terms of a model proposed by Spurway (1965). It was suggested that decreased anion adsorption at the cell membrane, a function of increased anion hydration, led to a reduced charge at the anion 'channels' and thus to the increased chloride flux. Such a model might apply to the ascites cell.

Comprehensive studies of permeability changes resulting from anion substitution have been made on the erythrocyte, a cell characterized by enormously high chloride permeability. Recent work in this area is that of Passow (1969), Wieth (1970) and Gunn & Tosteson (1971). Anion selectivity was postulated to arise from an exchange with anions which were bound to fixed positive membrane sites. According to this concept an anion which binds very readily to the fixed positive sites will enhance its own exchange and diminish the exchange of other anions present in the medium (Wieth, 1970). Passow (1969) has shown that at a given pH, progressive replacement of external chloride with sulphate increased the steady-state sulphate flux across erythrocytes. He interpreted these

results in terms of 'a Cl/SO_4 competition'. By inference, the *chloride* flux would be diminished in such sulphate media. In contrast, sulphate *increased* chloride exchange in the ascites cell. Therefore, the basic mechanism of anion exchange may differ in these two cells. As Passow (1969) has pointed out, however, the positive fixed charge hypothesis may not have universal applicability. Indeed, a difference in the basic mechanism of anion exchange would be consistent with a considerable difference in anion selectivity. Thus the erythrocyte is a million times more permeable to anions like chloride than to sodium and potassium cations (Tosteson, 1959) but the ascites cell has a rate coefficient of cell chloride exchange which is only ten times greater than the potassium rate coefficient (Hempling, 1962) and is actually less than the sodium fast compartment coefficient of cell exchange (Aull & Hempling, 1963).

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